

Safety of sputum induction in chronic obstructive pulmonary disease

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ABSTRACT: The aim of the present study was to evaluate the safety of sputum induction in patients with varying severity of chronic obstructive pulmonary disease.

The subjects were 28 smokers with baseline forced expiratory volume in one second (FEV₁) of (mean and range) 1.8 (0.8–2.9) L that is 53 (28–69)% of the predicted and reversibility of 2.5 (–7.4–9.9)%. Sputum was induced after premedication with 200 µg salbutamol at increasing concentrations (0.9, 3, 4, and 5%) of hypertonic saline nebulized by an ultrasonic nebulizer.

The procedure was well tolerated, and none of the patients reported major side-effects. However, the mean change from prebronchodilator FEV₁ during induction was –8.5 (–23–11)%, $p=0.001$, and from postbronchodilator FEV₁ –10.7 (–25–5)%, $p<0.0001$. Three (11%) of the patients had a fall in FEV₁ from the prebronchodilator baseline of >20%, and a further 10 (36%) had a fall of 10–20%. Patients with greater reversibility in airway obstruction seemed to get the best benefit from the bronchodilator pretreatment, since there was an inverse relationship between reversibility in FEV₁ and fall in FEV₁ during induction ($r=-0.4$, $p=0.03$).

It is concluded that sputum induction by hypertonic saline inhalation can cause meaningful bronchoconstriction in patients with chronic obstructive pulmonary disease, despite pretreatment with an inhaled β_2 -agonist. The results highlight the importance of monitoring spirometry during sputum induction to detect bronchoconstriction.

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Sputum induction by inhalation of hypertonic saline has been used as a direct and relatively noninvasive method to investigate airway inflammation. It has been applied in patients with asthma and chronic obstructive pulmonary disease (COPD) [1–4]. Sputum-induction methodology may offer important benefits in diagnosing patients with airflow obstruction and in comparing treatment strategies.

Although no standardized method for the induction of sputum has been agreed upon, recently a consensus on the use of sputum induction in asthma has appeared [5]. The challenge procedure should be performed in a standardized manner, because hypertonic saline can cause asthmatics to suffer from airway constriction. This procedure includes spirometry before and during the induction as well as pretreatment with a short-acting β_2 -agonist.

Some studies assess the safety of sputum induction in patients with asthma [6–8], but information on patients with COPD is scarce [3, 4]. The aim of the present study was to evaluate the safety and success of sputum induction in patients with varying severity of COPD [9].

Materials and methods

Subjects

The authors studied 28 patients with at least a 2-yr clinical diagnosis of symptomatic COPD [10] and a smoking history of 20–103 pack-yrs (table 1). Two (7%)

of them were exsmokers, all others were current smokers. Patients had to fulfil the following criteria: 1) prebronchodilator forced expiratory volume in one second (FEV₁) of <70% of the predicted [11]; 2) <10% reversibility from prebronchodilator FEV₁ to short-acting β_2 -agonist (200 µg of salbutamol). Patients treated for disease exacerbation or needing antibiotics for respiratory-tract infections during 6 weeks prior to the study were excluded.

FEV₁ (mean and range) was 1.8 (0.8–2.9) L, that is 53 (28–69)% pred and FEV₁/forced vital capacity was 52 (33–67)%. Reversibility to 200 µg of inhaled salbutamol was 2.5 (–7.4–9.9)% (table 1). Four (14%) patients used inhaled steroids (beclomethasone or budesonide, mean dose 900 µg·day⁻¹, range 800–1200 µg·day⁻¹) and two (7%) oral theophylline. None had regular oral steroid treatment. Five (18%) received short-acting β_2 -agonists and three (11%) anticholinergics as a rescue medication. Only one (4%) showed clinical evidence of allergy or atopy.

The study was approved by the ethics committee of Helsinki University Central Hospital (Helsinki, Finland), and all subjects gave their informed consent.

Study design

Two weeks before sputum induction, spirometry was performed and reversibility in FEV₁ was assessed (Spirotrac III; Vitalograph, Maids Moreton, UK). For FEV₁, the

Table 1. – Characteristics of subjects

n	28
Age	60 (51–68)
Male	19 (68%)
Smoking pack-yrs	52 (20–103)
Inhaled steroids	4 (14%)
Symptom score*	6.4 (3.3–11)
Δ PEF % [‡]	8.9 (1.9–39)
Baseline FEV ₁ L	1.8 (0.8–2.9)
Baseline FEV ₁ % pred	53 (28–69)
Baseline FVC L	3.5 (1.9–5.3)
Baseline FEV ₁ /FVC %	52 (33–67)
Reversibility in FEV ₁ %	2.5 (-7.4–9.9)

Data expressed as mean and range or number and percentage. FEV₁: forced expiratory volume in one second; FVC: forced vital capacity. *: average sum of three symptoms (cough, sputum-production, shortness of breath) per day, see *Methods* section. [‡]: average diurnal peak expiratory flow (PEF) variation during 1-week follow-up, see *Methods* section.

greatest accepted difference between the two highest values among three or more technically acceptable determinations was 4% [12]. During 1–2 weeks time before sputum induction, patients measured their peak expiratory flow (Mini Wright peak flow meter; Clement Clarke Int., London, UK) every morning and evening. They also recorded daily symptoms (cough, sputum-production, shortness of breath) on a scale ranging 0–4 (table 1). Patients were allowed to continue their usual medication for COPD, with the exception of long-acting bronchodilator therapy, which was not allowed for the 2 weeks prior to sputum induction. Short-acting bronchodilators were not allowed 4 h prior to induction.

Sputum induction

Sputum was induced by the method first described by PIZZICHINI *et al.* [9]. Before induction, spirometry was performed, after which 200 µg of salbutamol was given by metered dose inhaler. The inhalation technique was checked and medication was given under supervision of a trained nurse. Ten minutes later postbronchodilator spirometry was obtained. If the postbronchodilator FEV₁ was <1 L, only 0.9% hypertonic saline was used, and FEV₁ was checked every 3 min. If the postbronchodilator FEV₁ was >1 L, induction was started with 3% hypertonic saline, and FEV₁ was obtained every 7 min. The inhalation was stopped if FEV₁ dropped by > 20%, if bothersome symptoms occurred, or when the sputum sample was adequate. The inhalation was followed by postinduction spirometry. Duration of inhalation was calculated as time required to reach the end of inhalation or to decrease FEV₁ by >20%. The hypertonic saline solution was nebulized with an ultrasonic nebulizer (Ultra-neb 2000; DeVilbiss Health Care Inc., Somerset, PA, USA); (output 2.5 mL·min⁻¹, particle size 4.5 µm).

Sputum processing

The authors used the method of sputum examination of PIZZICHINI *et al.* [13]. Briefly, all sputum macroscopically free of salivary contamination was selected and treated with dithiothreitol (Sputolysin 10%; Calbiochem Corp., San Diego, CA, USA) and phosphate-buffered saline.

The suspension was centrifuged, and the supernatant was aspirated and stored in microfuge tubes at -20°C for later assay. The cell pellet was resuspended, and the absolute number of cells per milligram of processed sputum was calculated. Coded cytopspins were prepared and stained by May-Grünwald Giemsa stain and by toluidine blue in order to obtain a cell differential count. The sputum sample was considered adequate if it had <80% squamous epithelial cell contamination from saliva. The results are expressed as percentage of individual cells of the total nonsquamous cell count.

From sputum supernatant the concentrations (µg·L⁻¹) of eosinophil activation marker eosinophil cationic protein (ECP), and the neutrophil activation marker, myeloperoxidase (MPO), were measured using commercially available immunoassay kits (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

Data analysis

The authors calculated the change in FEV₁ from the prebronchodilator and from the postbronchodilator baseline. Data are expressed as mean and range. Comparisons of different patients groups were analysed by the Mann-Whitney U-test and Chi-squared test, when appropriate. Correlation was analysed by Spearman's rank correlation test. Wilcoxon's test for paired data was used to analyse changes in the same patient. Two-tailed p-values <0.05 were considered significant.

Results

The sputum induction procedure was well tolerated, and no patients reported major side-effects. An adequate sputum sample was obtained from 27 (96%) patients. The mean change from the prebronchodilator FEV₁ during induction was -162 (-541–205) mL that is -8.5 (-23–11)%, p=0.001, and from the postbronchodilator FEV₁ -202 (-625–95) mL that is -10.7 (-25–5)%, p<0.0001 (table 2, fig. 1). Three (11%) of the subjects had a fall in FEV₁ from the prebronchodilator baseline value of >20%, and a further 10 (36%) had a fall of 10–20%. All patients with a fall in FEV₁ of ≥20% were followed carefully and treated with inhaled salbutamol.

Table 2. – Sputum-induction data

	Patients n=28
Prebronchodilator	
Baseline	
Change in FEV ₁ mL	-162 (-541–205)
% change in FEV ₁ ⁺	-8.5 (-23–11)
Postbronchodilator	
Baseline	
Change in FEV ₁ mL	-202 (-625–95)
% change in FEV ₁ [‡]	-10.7 (-25–5)
Final saline concentration	
0.9%	3 (11%)
3%	21 (75%)
4%	4 (14%)
5%	0 (0%)
Induction time	8.3 (2–21)

Data expressed as mean and range or number and percentage. FEV₁: forced expiratory volume in one second. ⁺: p=0.001; [‡]: p<0.0001.

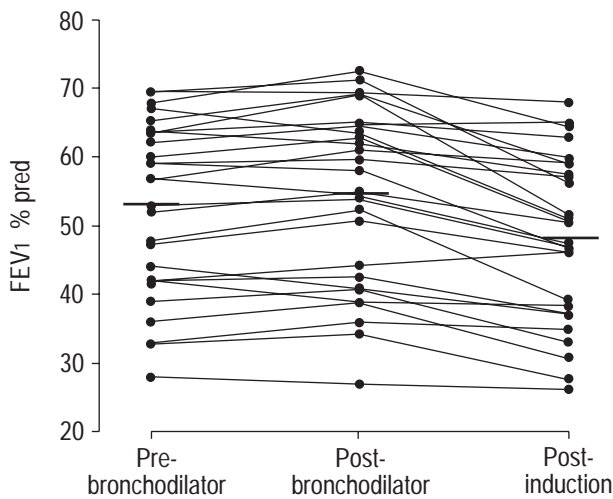


Fig. 1. – Forced expiratory volume in one second (FEV₁) as measured before and after salbutamol pretreatment and after sputum induction. Prebronchodilator FEV₁ increased significantly after salbutamol ($p=0.03$, Wilcoxon's test). During induction FEV₁ fell significantly ($p=0.001$ from the prebronchodilator baseline, $p<0.0001$ from the post-bronchodilator baseline). Horizontal bars represent the mean of all samples.

Three female patients (11%) had a postbronchodilator FEV₁ of <1 L, 0.87 (0.81–0.94) L, 36 (34–38)% pred, and sputum was induced using 0.9% saline only. The mean change in FEV₁ in these patients was -60 (-118–53) mL, that is -7.2 (-15–7)% from the prebronchodilator baseline, and -120 (-188–17) mL, that is -13.7 (-20–2)% from the postbronchodilator baseline.

The sputum total and differential cell counts as well as ECP and MPO results are shown in table 3. A statistically significant inverse relationship existed between reversibility in FEV₁ and fall in FEV₁ during induction, ($r=-0.4$, $p=0.03$; fig. 2). Otherwise, the authors could find no correlations among sputum markers and baseline clinical characteristics and change in FEV₁ during induction. No significant differences existed in any of the parameters between females and males or between patients on inhaled steroids and steroid naive-patients.

Discussion

It was found that 11% of the patients with COPD had a fall in FEV₁ of >20% during the induction procedure and another 36% a fall in FEV₁ of 10%–20%. The mean fall from the prebronchodilator baseline FEV₁ during induction

Table 3. – Sputum inflammatory markers

Sputum total cell count $10^3 \cdot \text{mg}^{-1}$	1.9 (0.09–15)
Eosinophils %	2.0 (0–11)
Neutrophils %	62 (38–89)
Lymphocytes %	0.4 (0–4)
Macrophages %	36 (9–62)
Bronchial epithelial cells %	0.10 (0–4.0)
Squamous epithelial cells %	6.8 (0–35)
Metacromatic cells %	0.10 (0–1.0)
ECP $\mu\text{g} \cdot \text{L}^{-1}$	1218 (36–4460)
MPO $\mu\text{g} \cdot \text{L}^{-1}$	12320 (531–45540)

Data are expressed as mean with range in parenthesis. ECP: eosinophil cationic protein; MPO: myeloperoxidase.

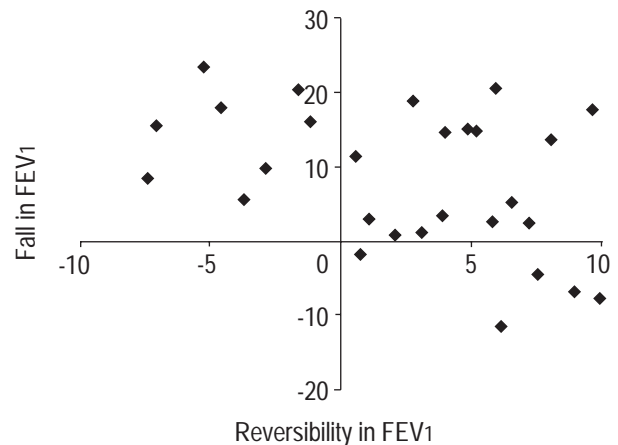


Fig. 2. – Relationship between reversibility in forced expiratory volume in one second (FEV₁) and fall in FEV₁ from prebronchodilator baseline during sputum induction, ($r=-0.4$, $p=0.03$, Spearman rank correlation).

was 8.5%, and from the postbronchodilator baseline 10.7%. However, all patients tolerated the sputum-induction procedure well and failed to report any major side-effects, although airway obstruction caused by the induction procedure did occur.

Pretreatment with a bronchodilator failed to prevent a fall in FEV₁, especially in patients with no or slight reversibility in FEV₁ caused by pretreatment salbutamol. The fall in FEV₁ during sputum induction was inversely correlated with reversibility in FEV₁. This indicates that COPD patients with more reversibility in FEV₁ may gain the best benefit from bronchodilator pretreatment against bronchoconstriction. Airway hyperreactivity and reversibility of airway obstruction are thought to be the characteristic features of asthma but can be seen in COPD as well. It has been shown that increased airway responsiveness in smokers is a risk factor for accelerated decline in FEV₁ over time and hence for the development of COPD [14].

The original studies introducing the sputum induction method reported few side-effects and no fall in FEV₁ [15, 16]. Studies concentrating upon safety aspects of sputum induction have found it safe even in patients with severe asthma [6–9] and in COPD [1–4]. Studies have proposed that sputum should not be induced in asthmatic patients with a postbronchodilator FEV₁ of <1 L or 60 % pred [5–7]. An aerosol of normal saline followed by hypertonic saline has, however, been safely used in patients with severe exacerbations of asthma [9]. The current authors applied a similar approach to their patients with a post-bronchodilator FEV₁ of <1 L, and the fall in FEV₁ was not significant.

It remains unclear whether the magnitude of bronchoconstriction depends on hypertonicity of the saline, the total amount inhaled, or the rate of delivery, or if it could be prevented by pretreatment with larger doses of inhaled salbutamol [17–19]. The current authors used a fairly high-output ultrasonic nebulizer (output $2.5 \text{ mL} \cdot \text{min}^{-1}$, particle-size $4.5 \mu\text{m}$). The original paper on asthmatics by PIN *et al.* [15] used a relatively low-output ultrasonic nebulizer (output $0.9 \text{ mL} \cdot \text{min}^{-1}$, particle-size $5.6 \mu\text{m}$), with which significant bronchoconstriction rarely occurred.

In conclusion, the authors have shown that sputum induction by inhalation of hypertonic saline can, in patients

with chronic obstructive pulmonary disease, cause bronchoconstriction. Patients whose airway obstruction is more reversible seem to be better protected by bronchodilator pretreatment. The results highlight the importance of monitoring spirometry during sputum induction in order to detect bronchoconstriction.

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